


Persistence of SARS-CoV-2 nasopharyngeal swab PCR positivity in COVID-19 convalescent plasma donors

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Abstract

Background: Nucleic acid persists after symptom resolution and infectivity for many viral infections via delayed clearance of nucleic acid fragments, non-infectious particles, or transmissible virus. For Coronavirus Disease 2019 (COVID-19), the relationship between nasopharyngeal (NP) swab positivity, the development of antibodies against COVID-19, and clinical history are unclear.

Study design and methods: Individuals who recovered from COVID-19 and volunteered to donate convalescent plasma (CP) were screened by NP swab PCR, responded to a questionnaire, and were tested for anti-COVID-19 antibodies.

Results: A proportion of 11.8% of individuals tested positive for SARS-CoV-2 by NP swab PCR greater than 14 days after the resolution of symptoms of active disease, including one donor who had asymptomatic disease and tested positive by NP swab 41 days after her initial diagnosis. Clinical history did not show a significant correlation with persistence of NP swab positivity. Also, NP swab positivity >14 days from symptom resolution did not correlate with anti-COVID-19 serology results. IgG anti-SARS-CoV-2 spike antibody strength correlated with hospitalization for COVID-19 using two different assays. Total anti-SARS-CoV-2 nucleocapsid antibody strength correlated with time from symptom resolution to sample collection and symptom duration.

Conclusions: SARS-CoV-2 nucleic acid is detectable long after the resolution of symptoms in a significant percentage of previously diagnosed individuals, which is important to consider when interpreting PCR swab results. Persistence of PCR positivity does not correlate with antibody strength or symptoms of COVID-19. If anti-spike antibody is used to assess CP potency, individuals who suffered severe COVID-19 disease symptoms may represent better donors.

1 | INTRODUCTION

Coronavirus Disease 2019 (COVID-19) convalescent plasma (CP) was widely proposed as a potential therapy for COVID-19 in the United States (US) during the initial months of viral spread within the country.^{1,2} Prior to

that, it was used to treat a limited number of COVID-19 patients in China, with suggestion of possible efficacy and no clear adverse effects.³ Furthermore, CP has been utilized during epidemics for nearly 100 years.⁴ A number of hospital-based and national blood collection centers, including the NorthShore University HealthSystem

Blood Bank, rapidly began convalescent plasma collection programs as an Institutional Review Board (IRB) approved clinical research program.

The use of convalescent plasma in the US was enabled by the US Food and Drug Administration (FDA) and the FDA recommendations for the collection of convalescent plasma changed quickly during the spring of 2020.⁵ Initially, the FDA stated that donors between 14 and 28 days of symptom resolution should test negative for SARS-CoV-2 PCR by testing either a nasopharyngeal (NP) swab or the blood product of the itself. Due to a lack of validated blood product PCR assays, most institutions either utilized NP swab or waited until after 28 days from symptom resolution before collecting plasma. Interestingly, many potential donors who were tested by NP swab remained positive >14 days from resolution of symptoms. Subsequently, the FDA changed their guidance to state that a confirmatory negative test was not necessary, even for donors who presented as early as 14 days after symptom resolution.

Similarly, the Center for Disease Control (CDC) established a “no-test” criteria to allow healthcare workers to return to work without confirmatory testing to ensure they are negative for COVID-19 by PCR.⁶ Since contact tracing in most states within the US remains underutilized, the possibility of low-level infectivity weeks after symptom resolution has not been completely excluded. Therefore, the significance of persistently positive SARS-CoV-2 NP swab remains unclear. Perhaps reassuringly, other viral infections are known to cause positive PCR tests, even after infectivity has ended.⁷ Conversely, there are reports of coronaviruses causing persistent asymptomatic infections in cats, with live and transmissible virus in some individuals.⁸ Additionally, asymptomatic respiratory coronavirus infections in humans are widespread and of unclear persistence.⁹

Due in part to early reports of potential asymptomatic transmission, the NorthShore CP collection study began testing all potential donors by NP swab PCR.¹⁰ This approach permitted investigation into the factors associated with persistent NP swab PCR positivity for SARS-CoV-2. In particular, we investigated whether donor characteristics or COVID-19 symptom history were associated with the duration of NP swab PCR positivity. Furthermore, we investigated whether serology test results correlated with clinical history or the persistence of NP swab PCR positivity.

2 | MATERIALS AND METHODS

2.1 | Donor recruitment

All participants contacted the NorthShore University HealthSystem Blood Bank to enroll in the IRB approved convalescent plasma collection study. The collection

program was listed on a national website for COVID-19 CP (www.ccpp19.org) and physicians within the NorthShore University HealthSystem were notified of the collection program by professional staff memo, with instructions on how to direct patients to the blood bank.

Potential participants were screened and scheduled by phone to ensure a history of a positive SARS-CoV-2 test result (PCR or serology) and >14 days from complete resolution of symptoms of active disease, which included (at least) extreme fatigue, cough, sore throat, fever, and diarrhea. Persistent loss of taste and/or smell was not considered a symptom of active disease. Phone screening involved notification that CP donors must also satisfy routine blood donor screening criteria, however a detailed donor history screening was not done by phone.

When donors presented for consent, they were asked to fill out a form that included a free-text report of COVID-19 symptoms and demographic information. Free text results were grouped into numerical variables of age, symptom duration, days from symptom resolution to sample collection, and maximum temperature (if febrile and measured). The categorical variables of initial NP swab result and sex were analyzed, along with variables related to COVID-19 disease symptoms: fever, loss of taste/smell, diarrhea, chills, aches, fatigue, night sweats, headaches, cough, sore throat, shortness of breath, chest tightness, other respiratory symptoms, gastrointestinal symptoms, skin symptoms (rash or hives), hospitalization, receiving treatment, history of autoimmune disease, history of past drug reactions, and history of bleeding or clotting disorder. Symptoms not reported in the free text fields were assumed to be negative/normal.

2.2 | Nasopharyngeal swab PCR

Participants were screened by rapid NP PCR approved under a US Food and Drug Administration Emergency Use Authorization (ID NOW, Abbott Laboratories, Chicago, IL). Swabs were tested immediately after sampling, without being placed in viral transport media. Individuals who tested positive by NP PCR were offered to be re-tested if they returned in 1-2 weeks.

2.3 | Serology testing

A subset of the total enrolled population had samples drawn for serology testing and sufficient volume available for serology testing using three in-house assays. These samples were collected from 214 donors. Anti-SARS-CoV-2 spike protein antibody testing was performed using the LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin Inc, Saluggia,

Italy) as well as the Access SARS-CoV-2 IgG assay (Beckman Coulter, Brea, CA). Anti-SARS-CoV-2 nucleocapsid protein antibody testing was performed using the Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, Basel, Switzerland). Importantly, the Roche assay tests for total (not isotype specific) anti-SARS-CoV-2 nucleocapsid reactivity. The quantitative cut-off index for each assay was used to assess antibody strength.

2.4 | Statistical analysis

To test for correlations between clinical history and persistence of NP swab PCR positivity, a per-swab analysis was performed by grouping swab results based on the number of days post symptom resolution when it was collected. Donors were not swabbed more often than once per week. All swabs taken greater or equal to 50 days from symptom resolution were grouped; one donor had two swabs within this group and the latter was used for analysis. Otherwise, all donors had only one swab per time period considered.

Unpaired student t-test with unequal variance was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA) to evaluate the statistical differences in numerical variables. Univariate analysis for associations between categorical variables were assessed using Fisher's exact test on a 2-by-2 contingency table in R (R Foundation for Statistical Computing, Vienna, Austria) using stats package v4, with a null hypothesis that the odds ratio is equal to 1 and alternative hypothesis that the odds ratio is not equal to 1. The Bonferroni correction was used to assess the statistical significance of p-values, therefore (given 20 tests performed, see Table 1) a p-value of less than or equal to 0.0025 was considered significant for the univariate analyses.¹¹

The relationship between serology results and NP swab positivity/clinical history was assessed using multivariate linear regression in R. In addition to the COVID-19 symptoms listed in Table 1, the model considered whether the individual was febrile (temperature > 100.4 F), sex, days post symptom resolution to serology test collection, initial NP swab result, age, and symptom duration. To avoid oversampling donors who were tested by NP swab more than once, only the first NP swab result for each donor was considered.

3 | RESULTS

3.1 | Donor characteristics

Between April 14, 2020 and June 9, 2020, a total of 272 donors enrolled the NorthShore University Health System convalescent plasma collection study. Median

TABLE 1 Demographics and clinical characteristics of convalescent plasma donors

Total donors, N	272
Age, median (range) y	44.9 (19-77)
Sex	
Male	130 (47.8%)
Female	142 (52.2%)
COVID-19 symptoms	266 (97.8%)
Cough	139 (51.1%)
Fatigue	138 (50.7%)
Muscle/body aches	132 (48.5%)
Loss of taste/smell	124 (45.6%)
Fever (temperature > 100.4 F)	111 (40.8%)
Headache	91 (33.4%)
Sore throat	63 (23.2%)
Chills	59 (21.7%)
Shortness of breath	56 (20.6%)
Diarrhea	52 (19.1%)
Other gastrointestinal symptoms	38 (14.0%)
Chest tightness	36 (13.2%)
Night sweats	21 (7.7%)
Skin rash or hives	12 (4.4%)
History of hospitalization for COVID-19	10 (3.79%)
History of treatment for COVID-19	27 (9.9%)
Azithromycin	9 (3.3%)
Hydroxychloroquine	7 (2.6%)
Tylenol	5 (1.8%)
Convalescent plasma	0 (0%)
Other	10 (3.7%)
History of drug reaction	25 (9.1%)
History of autoimmune disease	9 (3.3%)
History of bleeding or clotting	4 (1.5%)
History of use of convalescent plasma, N (%)	0 (0%)

Note: Symptoms are those experienced during COVID-19 disease. Other treatment included cough medicine, other antibiotics, and inhalers.

donor age was 47.8 years (range 19 to 77); 130 (47.8%) of the donors were male and 142 (52.2%) were female. The median time from symptom resolution to the initial NP swab test was 36.3 days (maximum 79 days), see Table 1.

3.2 | COVID-19 history

The median duration of COVID-19 symptoms was 13.6 days (range 0 to 65). Ten donors (3.7%) were

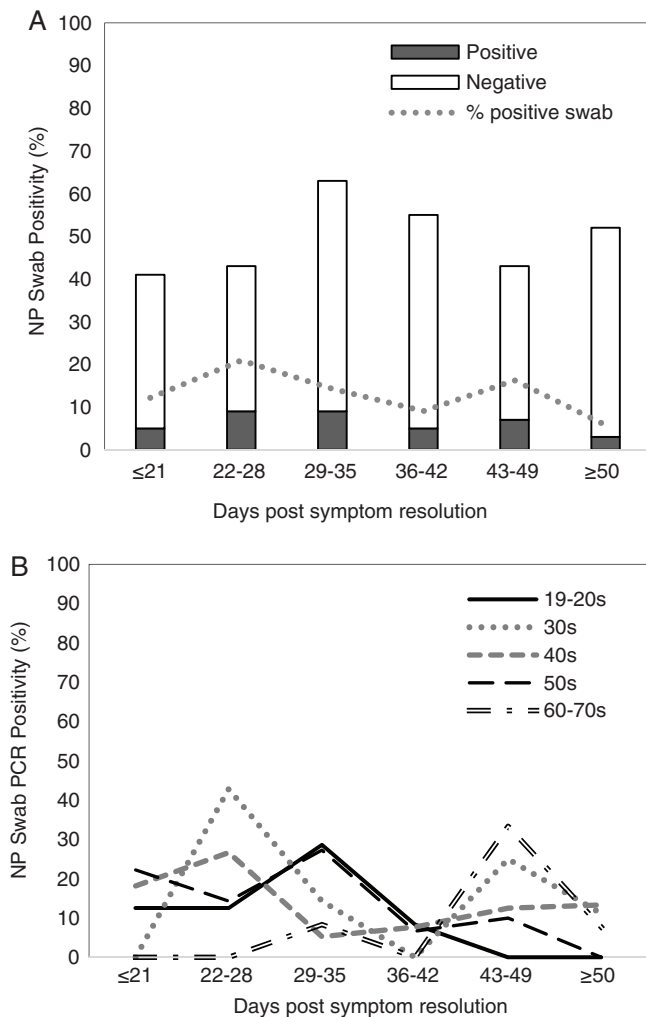


FIGURE 1 Nasopharyngeal (NP) swab positivity after symptom resolution. Percent positive NP swabs during each week-long period post symptom resolution is shown for the donor population overall (A) and broken down into donor age groups (B)

hospitalized for COVID-19, and none of them received a convalescent plasma for the therapy. Self-reported clinical symptoms of COVID-19 are shown in Table 1. Most of the donors described these symptoms as mild to non-severe. Six (2.2%) donors reported no symptoms of COVID-19.

3.3 | Persistence of NP swab PCR positivity

Initial NP Swab PCR was positive in 32 (11.8%) potential donors. The median time from symptom resolution to the initial positive swab was 31.8 ± 14.8 days. Donors who tested positive were offered serial follow-up with repeat NP swab in 7-14 days. By June 29, 2020 a total of 300 NP swabs were performed on these 272 donors. Six donors

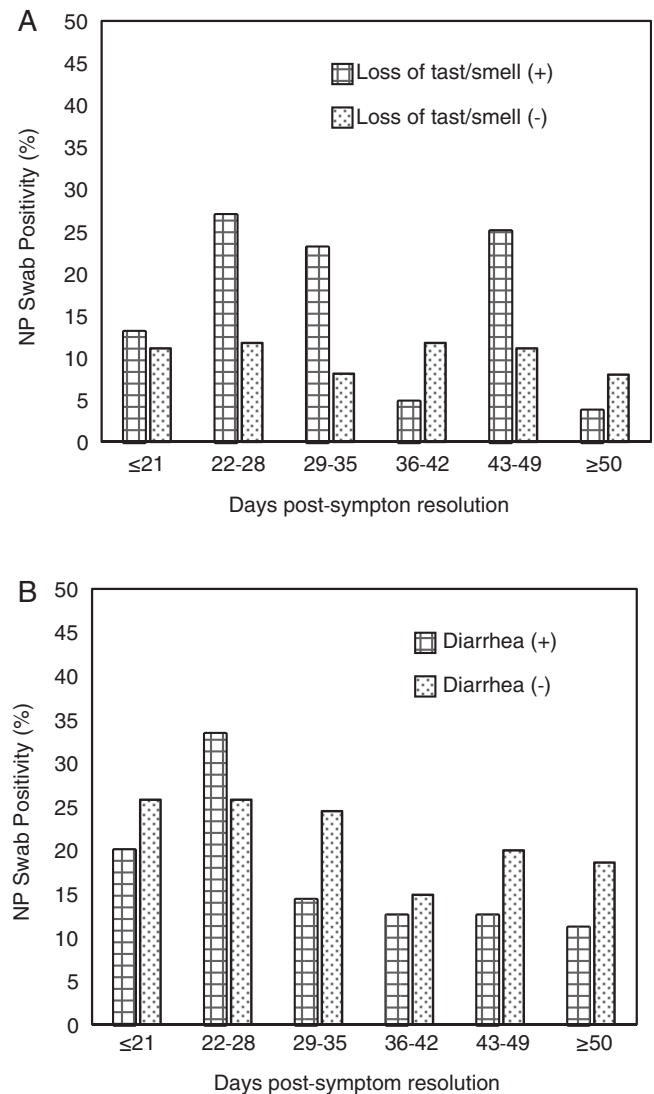


FIGURE 2 Diarrhea and Loss of Taste/Smell. Neither the presence nor absence of diarrhea or loss of taste/smell showed a statistically significant association with the positive NP swab rate during each time period (≤21, 22-28, 29-35, 36-42, 43-49, and ≥50 days post symptom resolution). For loss of taste/smell, *P* values were .28 and .14 during the 22-28 and 29-35 day time periods, respectively (Fisher exact test)

were positive initially and positive when they returned for a second swab; of those, four were negative when tested a third time. One donor remained positive by NP swab 75 days after the resolution of symptoms, after three consecutive positive swabs. Ten donors who tested positive initially did not return for a subsequent swab, while one donor positive on their second swab did not return for a third.

Analyzing NP swabs performed in the time periods of ≤21, 22-28, 29-35, 36-42, 43-49, ≥50 days after the resolution of symptoms, the positivity rate was 12.2%, 20.9%, 14.3%, 9.1%, 16.3%, and 5.8%, respectively (see

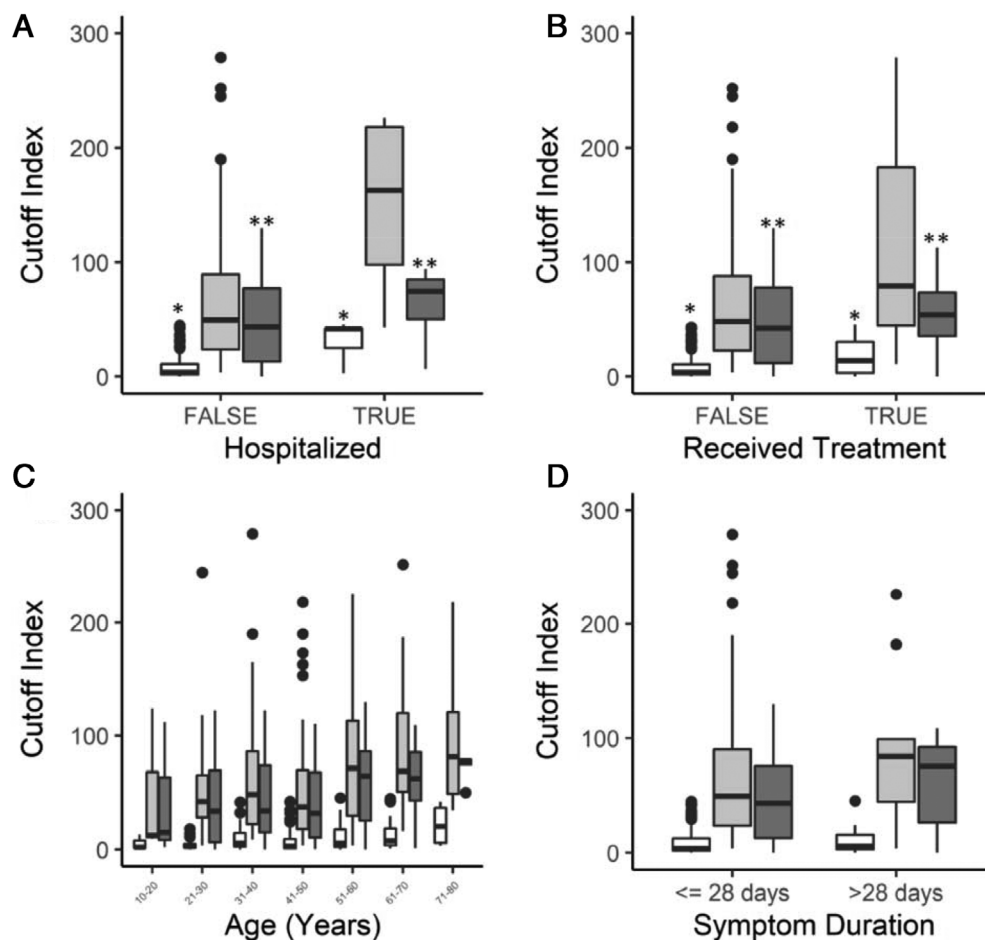


FIGURE 3 Correlation between serology results and donor factors. Cutoff indices are shown for Beckman SARS-CoV-2 IgG Antibody Assay (white), Roche SARS-CoV-2 Total Antibody Assay (light gray), and Diasorin SARS-CoV-2 IgG Antibody Assay (dark gray) relative to select patient characteristics. With the IgG assays, patients hospitalized or who received treatment had a statistically significant increase in cutoff index values, which was not seen with the total antibody assay (A and B). Increasing age was positively correlated with increasing cutoff indices for the Beckman IgG assay (C). Increasing symptom duration was found to be positively associated with increasing cutoff indices measured in the total antibody assay, but not for the IgG specific assays. Antibody reactivity for individuals with symptom duration less than versus greater than 28 days is shown in D

Figure 1A). One individual who did not have any symptoms of COVID-19 had a positive swab (41 days after diagnosis of COVID-19 by PCR).

Donor characteristics and COVID-19 symptom history (see Table 1) were compared between positive NP swabs and negative NP swabs within one-week time periods from symptom resolution to swab collection. There was no correlation between donor age and persistence of PCR positivity (Figure 1B). There was a slight correlation between loss of taste/smell and the persistence of NP swab positivity. On the other hand, there was a possible inverse relationship between the symptom of diarrhea and persistence of NP swab positivity (Figure 2). However, these correlations did not reach statistical significance after correcting for multiple observations.

Multivariate analysis of the serology test results revealed no significant correlation between antibody cut-off

index and whether a donor had a positive NP swab. The anti-nucleocapsid total antibody cut-off index was significantly higher for samples collected a greater number of days from symptom resolution ($P = .00007$) and samples from donors who had a longer symptom duration ($P = .001$). The Beckman anti-spike cutoff index was significantly higher for donors who had been hospitalized (0.00002), received treatment for COVID-19 ($P = .01$), and were older ($P = .02$). Similarly, the DiaSorin anti-spike cutoff index was higher for donors who had been hospitalized ($P = .007$). See Figure 3. For patients who were hospitalized for COVID-19, median anti-spike cutoff index was 32.0 ± 15.4 (Beckman) and 151.9 ± 73.7 (DiaSorin) versus 8.3 ± 9.8 and 64.3 ± 55.7 for non-hospitalized patients, respectively. The other factors tested did not show a statistically significant effect on serology results.

4 | DISCUSSION

Persistent infection with COVID-19 might be defined as active viral replication beyond the currently accepted post-exposure quarantine period of 14 days. It is unclear whether persistent PCR positivity by NP swab is due to persistent infection with transmissible virus or non-transmissible nucleic acid fragments. These two models may not be mutually exclusive across the population and future studies are needed to determine whether individuals persistently positive by NP swab PCR represent an infectious risk. One possible study could attempt to culture live virus from individuals with persistently positive NP swabs, however care must be taken interpreting negative culture results, since viral culture is known to have limited sensitivity.¹² Therefore, aggressive contact tracing for individuals with persistent PCR positivity might also be considered. It is possible that persistent low-level PCR positivity (at times below the detection limit our assays) may account for reports of COVID-19 recurrence.^{12,13}

With the current lack of data as to whether persistent NP swab PCR positivity correlates with persistent infectivity, there are two major safety concerns relative to convalescent plasma in particular. First, even if CP donors with persistent positivity are minimally infectious, the inability to maintain social distancing and the common desire to remove a face mask during donation makes the possibility of donor room transmission high. Second, SARS-CoV-2 is known to infect endothelial cells and a recent report of intra-utero transmission has emerged.¹⁴ Therefore, the possibility of transfusion transmitted SARS-CoV-2 cannot be completely disregarded and donors with persistently positive NP swabs may have a higher chance of transmitting virus in their plasma. Realistically, transfusion transmission of SARS-CoV-2 may be of greater concern if the plasma is used as a prophylaxis and provided to individuals who are not actually infected and therefore more vulnerable to transfusion transmitted disease. Perhaps pathogen inactivation should be considered when CP is used prophylactically.

Currently, healthcare organizations and other businesses are formulating plans to bring individuals back on-site, despite the lingering threat of COVID-19. Various strategies have been proposed to safely do this, some involving widespread testing of asymptomatic individuals. The data presented here as well as recent reports argue that the interpretation of widespread testing may be complicated by persistent PCR positivity, especially in individuals who were clinically asymptomatic and never diagnosed originally.¹⁵ It also raises the possibility that individuals who appear to be re-infected were actually just persistently positive.¹⁶ Ultimately, the issue becomes a practical one. For example, if a negative NP swab PCR

is needed for clearance for surgery, how long should a patient be made to wait if they have persistently positive NP swabs?

One potential weakness of study is the small number of donors tested. Larger and focused studies are needed to determine if loss of taste/smell is truly correlated with the persistence of NP swab positivity, although the data presented here argues against a robust correlation. Theoretically, both could be the result of higher viral load within the nasal mucosa. The trend for donors with diarrhea to have negative NP swab results similarly may reach statistical significance with additional data. If verified, these trends might suggest different manifestations of COVID-19 infection: gastrointestinal versus nasopharyngeal. Another potential weakness of this study is that the sensitivity of the rapid PCR assay used may have underestimated the prevalence of positive individuals.¹⁷ Finally, the cutoff indices used to assess antibody strength may not be linearly related to antibody reactivity; however, it is assumed that cutoff index is positively correlated with reactivity.

Importantly, multivariate analysis of clinical history, NP swab positivity, and serology results suggests that donors who were hospitalized (suggesting more severe COVID-19 disease) may provide more potent convalescent plasma, based on anti-spike antibody reactivity as a measure of antiviral potency.¹⁸ However, this study is limited in the number of individuals enrolled and the enrichment for individuals who had mild to moderate COVID-19, which is to be expected in a CP donor population during the early weeks of the epidemic. Further studies are needed to ensure that these findings hold true in larger cohorts and for longer follow-up intervals.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DISCLAIMERS

None.

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